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Original article

In silico modelling of the interaction of flavonoids with human P-glycoprotein nucleotide-binding domain

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Abstract

A three-dimensional model of human ABCB1 nucleotide-binding domain (NBD) was developed by homology modelling using the high-resolution human TAP1 transporter structure as template. Interactions between NBD and flavonoids were investigated using in silico docking studies. Ring-A of unmodified flavonoid was located within the NBD P-loop with the 5-hydroxyl group involved in hydrogen bonding with Lys1076. Ring-B was stabilised by hydrophobic stacking interactions with Tyr1044. The 3-hydroxyl group and carbonyl oxygen were extensively involved in hydrogen bonding interactions with amino acids within the NBD. Addition of prenyl, benzyl or geranyl moieties to ring-A (position-6) and hydrocarbon substituents (O-*n*-butyl to O-*n*-decyl) to ring-B (position-4) resulted in a size-dependent decrease in predicted docking energy which reflected the increased binding affinities reported in vitro.

Keywords: Homology modelling; ABCB1; Nucleotide-binding domain; Flavonoid

1. Introduction

The P-glycoprotein transporter, ABCB1, a member of the ATP binding cassette (ABC) super-family of transporters, is a xenobiotic efflux pump that limits intracellular drug accumulation by active extrusion of compounds out of cells. ABCB1 possesses broad substrate specificity and substrates include members of many clinically important therapeutic drug classes, including anti-HIV protease inhibitors, calcium channel blockers used in the treatment of angina and hypertension, antibiotics and cancer chemotherapeutics [1].

The expression of ABCB1 in pharmacokinetically important tissues, particularly the intestine and liver, and in blood–tissue barriers such as the blood–brain barrier [2], means the transporter is able to influence drug absorption, tissue distribution, elimination and excretion and consequently have a major impact on the pharmacokinetic profile of many therapeutic agents.

Although existing cancer chemotherapeutic approaches are being used more effectively, treatment options for recurrent or highly drug-resistant tumours are often ineffective [3]. ABCB1 is implicated in tumour multi-drug resistance (MDR), a phenomenon whereby cells become refractory to numerous structurally unrelated chemotherapeutic drugs. ABCB1 is responsible for the active cellular efflux of drugs within at least four of the major groups of naturally occurring chemotherapeutics (anthracyclines, vinca alkaloids, taxanes and epipodophyllotoxins) and hence interacts with chemotherapeutics that are used extensively [4–6]. ABCB1 activity (resulting in reduced absorption of orally administered drugs, decreased drug penetration into brain and decreased intracellular accumulation of chemotherapeutics in tumour cells) has clinical consequences such as reduced treatment efficacy and even failure of drug-based treatment regimes. Thus, a means of effectively inhibiting ABCB1 activity in patients receiving therapeutics known to be ABCB1 substrates is an attractive means of potentially enhancing therapeutic efficacy.

Fundamental to ABCB1-mediated cellular drug efflux is the ability of the transporter to bind and hydrolyse ATP. ABCB1,

Abbreviations: ABC, ATP binding cassette; CFTR, cystic fibrosis transmembrane regulator; HIV, human immunodeficiency virus; MDR, multi-drug resistance; NBD, nucleotide-binding domain; PDB, protein databank; QSAR, quantitative structure activity relationship.

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as with many ABC transporters, possesses an architecture of intracellular nucleotide-binding domains (NBDs) and transmembrane domains. The NBDs contain three distinct motifs, Walker A, Walker B and the Signature or C motif. The Walker A motif, also known as the P-loop, interacts with the phosphates of nucleotide di- and tri-phosphates. The function of the Walker B sequence is less clear, but it is thought to be involved in Mg^{2+} coordination within the transporter and in polarising water molecules, the activating species for hydrolysis [7]. The precise function of the Signature motif is unknown. However, mutations within this region lead to loss of transport and it is thought the motif is important in conducting the energy released from ATP hydrolysis to the transmembrane domains, thereby producing a conformational change leading to translocation of substrates across the membrane [8]. It has also been postulated that the Signature motif may function as a γ-phosphate sensor, detecting the presence of the γ-phosphate of ATP in the opposing monomer within a dimeric NBD structure [9].

Thus, since ATP binding and hydrolysis within NBDs are crucial for maintaining ABCB1-mediated drug translocation, disruption of these processes is potentially a powerful means of inhibiting transporter activity.

Plant polyphenols, specifically flavonoids (Fig. 1), occur in abundance in many of the foods and drinks that are consumed on a regular basis.

They are found in high levels in fruits, vegetables, nuts, wine and tea and intake can be up to several hundred milligrams a day. A number of studies documenting their use have reported positive effects on human health due to their antioxidant, anti-inflammatory, antiviral and anticancer properties [10]. Flavonoids have been shown to interact with NBDs of ATPase transporters and inhibit ATPase activity [11–15] and direct binding of flavonoids to the C-terminal NBD (NBD2) of murine Abcb1 has been demonstrated using saturation transfer difference-NMR spectroscopy [16]. Thus, NBDs represent potential targets for therapeutic intervention and the use of flavonoids as potential modulators of ABCB1-mediated drug efflux is highly attractive.

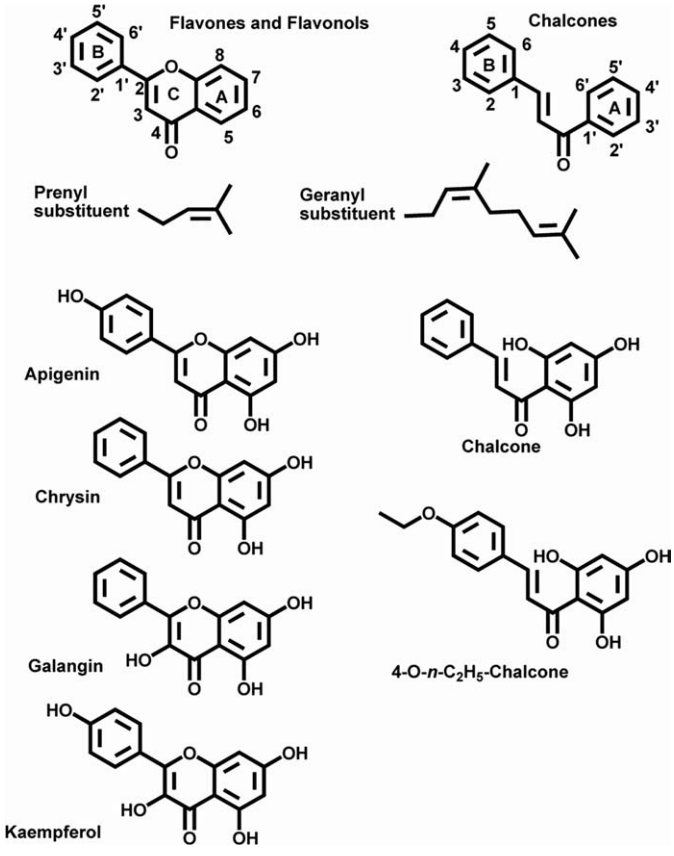

Fig. 1. Structure of flavonoids.

In silico screening of chemical entities represents a powerful and rapid approach for identification and selection of potential lead compounds prior to in vitro and in vivo studies. However, at present, the crystal structure of ABCB1 is available at a resolution of only 8 Å [17], which is insufficient to allow analyses of the mechanism of interaction of flavonoids with ABCB1 NBDs. To address this we have generated an in silico homology model of the C-terminal ABCB1 NBD (NBD2) of human P-glycoprotein and correlated the docking characteristics of a series of flavonoids and flavonoid-derivatives with the findings of in vitro functional studies and crystallographic data.

2. Results

2.1. Template identification

A BLAST search filtered for known 3D structures indicated that, to date, NBD2 of the human ABCB1 transporter demonstrated greatest homology with the C-terminal NBD of the human TAP1 transporter (PDB code: 1JJ7) [18]. Over the 249 amino acids comprising the NBD there was 46% sequence identity between ABCB1 and TAP1 (Fig. 2).

The NBD of *E. coli* haemolysin B (PDB code: 1MT0) [19], a protein transporter; the *L. lactis* MDR ABC transporter NBD (PDB code: 1MV5) [20]; the *S. typhimurium* histidine permease transporter NBD (PDB code: 1B0U) [21] and the *M. jannaschii* MJ0796 ABC transporter NBD (PDB code: 1L2T) [22] possessed between 25% and 46% homology with human ABCB1 NBD2 and were employed as templates for homology modelling since it is generally accepted that a sequence identity above 25% leads to reasonable homology models [23].

2.2. Template sequence homology and model secondary structure

Multiple sequence alignment revealed a high level of homology between ABCB1 NBD2 and the NBDs of other ABC proteins, particularly within the Walker A, Walker B, Signature sequence and Q-loop regions (Fig. 2). Within all template sequences Gly1070, Gly1073 and the GKST (1075–1078) residues of the Walker A sequence were invariant, as were SGGQ (1177–1180) Gln1182, ARA (1187–1189) within the Signature sequence and Asp1200, Thr1203, Leu1206 and Asp1207 residues within the Walker B motif.